OPERATIONAL AND SCIENTIFIC NOTES

ASSESSMENT OF *AEDES SIERRENSIS* AS A VECTOR OF CANINE HEARTWORM IN UTAH USING A NEW TECHNIQUE FOR DETERMINING THE INFECTIVITY RATE

GLEN A. SCOLES,[1] SAMMIE L. DICKSON[2] AND MARK S. BLACKMORE[1]

ABSTRACT. Both *Aedes sierrensis* and *Dirofilaria immitis* have recently become established in Utah. We evaluated the vector potential of this *Aedes sierrensis* strain using a new technique for detecting *Dirofilaria immitis* in individual mosquitoes. Survival of *Aedes sierrensis* females after bloodfeeding did not differ from that of *Ae. triseriatus* but infective *Ae. sierrensis* produced significantly more L_3 nematodes. This observation and epidemiological data support the hypothesis that *Ae. sierrensis* is the vector of canine heartworm in Utah. Infectivity was determined by counting infective-stage parasites that migrated into the medium after individual mosquitoes were decapitated or crushed in the wells of tissue culture plates. Complete recovery of infective-stage nematodes was attained in 60–74% of the mosquitoes and 77–93% of all L_3 were collected with this technique. There were few false negatives. High recovery rates (mean = 89%) were also obtained for mosquitoes treated en masse.

Canine heartworm, *Dirofilaria immitis*, is widespread in the United States. Although more than 60 mosquito species have been identified as potential vectors, there have been relatively few field isolations of *D. immitis* third-stage larvae (L_3) from mosquitoes in the U.S. (Grieve et al. 1983). The usual methods for detecting infective *D. immitis* in mosquitoes involve time and labor intensive dissections of individual insects or en masse processing that masks important epidemiological factors such as parasite prevalence and infectivity. We have developed an alternative technique for rapidly assessing the infectivity of individual mosquitoes. This paper presents details of this new technique, its efficiency relative to other methods, and evidence linking *Aedes sierrensis* (Ludlow) to the recent establishment of canine heartworm in Salt Lake City.

The annual incidence of *D. immitis* infections in Utah has increased steadily since indigenously transmitted *D. immitis* was first diagnosed there during 1987 (Marshall 1990). Although canine heartworm is endemic in northern California (Walters and Lavoipierre 1984) and west-central Colorado (Sears et al. 1980), it was not believed to be established in Utah prior to 1987. Earlier reports of canine heartworm in Utah were sporadic and all involved dogs with histories of prior residence in endemic areas of other states. By 1991, 25 canine heartworm cases were reported in Utah, including the first within Salt Lake City (Erekson 1991).

The sudden appearance and increasing incidence of *D. immitis* in Utah coincided with the invasion and establishment of the western tree hole mosquito, *Ae. sierrensis*, in the state. *Aedes sierrensis* was first discovered in northern Utah in 1965 (Nielsen et al. 1967) but was not detected in Salt Lake City until 1987 when a single adult was collected in a Salt Lake City Mosquito Abatement District (MAD) light trap. This was the first time *Ae. sierrensis* had been collected in the district's 68 years of mosquito surveillance. *Aedes sierrensis* was firmly established by 1991 and accounted for 20% of all complaints handled by the Salt Lake City MAD from April through mid-September of that year (Hatch and Dickson 1991).

At least 2 studies have implicated *Ae. sierrensis* as a vector of heartworm in northern California. Weinmann and Garcia (1974) found that *Ae. sierrensis* collected as larvae would feed on an infected dog and the parasites developed to the infective L_3 stage. Similar results were obtained by Walters and Lavoipierre (1982), who showed that *Ae. sierrensis* females were attracted to and fed upon an infected dog in a kennel trap. They confirmed that *D. immitis* develop to the infective stage in these mosquitoes. Neither study demonstrated parasite transmission by *Ae. sierrensis* nor has there been any report of heartworm-infected adults of this species collected in nature.

To test the vector potential of the Salt Lake City strain of *Ae. sierrensis*, we fed mosquitoes on a *D. immitis*-infected dog. For these studies, larvae were collected from tree holes in Salt Lake City during April 20–24, 1992, transported to Notre Dame on ice and reared to adults in enamel pans under constant temperature (22.5°C) and photoperiod (16L:8D). Female mosquitoes were fed on a heartworm-infected dog at 7–14 days post-eclosion and held 15 days (23°C; 70–80% RH) to allow parasite development to L_3. For comparison, we also tested *Ae. triseriatus* (Say) (WALTON strain).

The presence of infective-stage nematode larvae was assessed using the following simple, rapid technique for "dissecting" large numbers

[1] Vector Biology Laboratories, University of Notre Dame, Notre Dame, IN 46556.

[2] Salt Lake City Mosquito Abatement District, P.O. Box 16189, Salt Lake City, UT 84116.

Table 1. Recovery of *Dirofilaria immitis* third stage larvae (L_3) from infective mosquitoes using the decapitation method.

Species	% with complete L_3 recovery	% of total L_3s recovered	% false negatives
Ae. triseriatus	61.0 (14/23)	77.5 (69/89)	0
Ae. aegypti	70.0 (7/10)	92.8 (77/83)	0
Ae. albopictus	74.0 (29/39)	88.1 (163/185)	5.0

Table 2. Yields of third stage larvae (L_3) of *Dirofilaria immitis* from mosquitoes using 2 different dissection techniques.

Species	Technique	No. L_3s per infective mosquito ± SD	n	Significance
Ae. albopictus	Decapitation	6.09 ± 4.3	22	
	Crushing	7.54 ± 5.9	22	$U = 269$, n.s.
Ae. triseriatus	Decapitation	8.68 ± 5.0	22	
	Crushing	8.25 ± 6.9	12	$U = 148$, n.s.

of individual mosquitoes. Mosquitoes were removed from rearing cages with a vacuum aspirator, immobilized by chilling and placed in a petri dish on ice. An individual mosquito was placed into each well of a 24-well tissue culture plate and carefully decapitated with fine forceps. Each well contained 1 ml of RPMI 1640 tissue culture medium (Sigma) with 10% iron supplemented calf serum (Sigma) and antibiotics. We found that phosphate buffered saline (PBS) could be used as a low-cost substitute for the tissue culture medium when screening large numbers of mosquitoes. The tissue culture plates were placed in a 37°C incubator for 90 min to allow time for the L_3s to migrate into the medium. The wells were examined at 40× on an inverted microscope. Mosquitoes were scored as infective if L_3s migrated into the medium.

Survival to 15 days post-blood-feeding did not differ significantly between *Aedes sierrensis* (83%) and *Ae. triseriatus* (63%) ($G = 2.51$; $P > 0.05$), but infective *Ae. sierrensis* produced significantly more L_3s than *Ae. triseriatus* ($U = 214.5$, $P < 0.05$). Of the 39 *Ae. sierrensis* females that survived, 85% were infective; these produced an average of 4.24 L_3s per infective mosquito (S.D. = 2.7; $n = 33$). In comparison, 90% of *Ae. triseriatus* were infective and produced 2.33 L_3s per mosquito (S.D. = 1.5; $n = 9$).

Further studies were carried out using laboratory strains of *Ae. aegypti* (LIVERPOOL), *Ae. triseriatus* (WALTON) and *Ae. albopictus* (U.S. strains) to evaluate the recovery efficiency of this technique. Mosquitoes were fed on the infected dog and processed as described above. After allowing time for the L_3s to migrate out, each mosquito was dissected completely to determine how many L_3s remained in the head and body. The efficiency of this technique is shown in Table 1. There was complete recovery of L_3s in 60–74% of the infective mosquitoes. Overall, 77–93% of the total L_3s present were collected and there was an extremely low rate of false negatives. In *Ae. aegypti* (Linn.) and *Ae. triseriatus*, L_3s migrated out of all of the infective mosquitoes. In *Ae. albopictus* 95% of all mosquitoes with L_3s were detected with this method (5% false negative).

We also modified the technique for en masse dissection and compared the efficiency of this method to that of individual dissection. Four groups of *Ae. albopictus* (2 INDIANAPOLIS; 2 NEW ORLEANS) were crushed in wells of a 6-well tissue culture plate. Each well contained 5 ml of culture medium and 6–15 mosquitoes. The mosquitoes were crushed with the blunt end of a glass rod and the plates were placed in the incubator. After allowing 90 min for the L_3s to migrate out, the mosquitoes were dissected further and the number of remaining L_3s counted. The proportion of total L_3s recovered using en masse dissection ranged from 78 to 100% with a mean of 89%.

Finally, we compared the L_3 yields from decapitated mosquitoes versus crushed specimens using 2 groups of *Ae. albopictus* (NEW ORLEANS) and 2 groups of *Ae. triseriatus*. One group of each species was placed in wells with tissue culture medium (one mosquito per well) and carefully decapitated with forceps. The other groups were set up in the same way in PBS but the individuals were crushed as described above. There was no significant difference (Mann-Whitney U; $P > 0.05$) in number of L_3s per infective host obtained by either method (Table 2).

Although the en masse dissection technique using a Baermann funnel has been described

previously (Muller and Denham 1974), it has been used in field studies on only a few occasions (Christensen and Andrews 1976, Arnott and Edman 1978, Lewandowski et al. 1980). Modifications of this method have been applied in harvesting larvae mass-produced for *in vitro* studies (Ash and Riley 1970). The main advantages of these techniques are speed and high recovery rates of 91–97% (Muller and Denham 1974). However, because infection data are not available for individual mosquitoes, these techniques only allow calculation of "relative abundance" (i.e., total no. L_3s per total no. mosquitoes). With individual dissection data, the proportion of the mosquito population that is infective and the average number of L_3 per infective mosquito can be determined. These data are important for assessing transmission probability from biting rates, and neither can be determined using en masse dissection. Our dissection method allows rapid collection of data for individual mosquitoes, recovery rates similar to the en masse method reported by Muller and Denham (1974), and a very low proportion of false negatives. Our method detects only infective individuals. A mosquito may be "infected" without ever becoming "infective." This distinction should be considered if the technique is to be used for surveys of natural populations. Our technique is obviously applicable to other filarid/vector systems.

Aedes sierrensis from Salt Lake City was highly susceptible to *D. immitis* infection. Not only was the proportion of infective individuals high (85%), but they produced nearly twice as many L_3s per infective mosquito as the laboratory strain of *Ae. triseriatus* under the same conditions. The infectivity rate also was high relative to other species we have tested (unpublished data). High fecundity (Hawley 1985), limited migration distance (Bennett 1980), high susceptibility and survival following infection with *D. immitis* combine to make *Ae. sierrensis* a potentially important vector of canine heartworm. The arrival of *Ae. sierrensis* in Salt Lake City coincident with the first occurrence of canine heartworm cases strongly implicates this species as the vector of the parasite in Utah.

We thank G. B. Craig, Jr., and C. C. Lord for reviewing drafts of this manuscript, and B. A. Mullens for bibliographic assistance. The comments of 2 anonymous reviewers also improved the manuscript. This work was supported in part by NIH Parasitology/Vector Biology Training Grant #5 T 32 AI 07030 and NIH Research Grant # AI 02753 (to G. B. Craig, Jr.). The dog used in this research was housed in the Freimann Life Science Center, University of Notre Dame, and cared for following guidelines set forth in the "Guide for Care and Use of Laboratory Animals," Institute for Laboratory Animal Resources, National Research Council.

REFERENCES CITED

Arnott, J. J. and J. D. Edman. 1978. Mosquito vectors of dog heartworm, *Dirofilaria immitis*, in western Massachusetts. Mosq. News. 38:222–230.

Ash, L. R. and J. M. Riley. 1970. Development of *Brugia pahangi* in the jird, *Meriones unguiculatus*, with notes on infections in other rodents. J. Parasitol. 56:962–968.

Bennett, S. R. 1980. Dispersal of the western tree hole mosquito, *Aedes sierrensis* (Diptera: Culicidae), in an orchard habitat. J. Med. Entomol. 17:156–164.

Christensen, B. M. and W. N. Andrews. 1976. Natural infection of *Aedes trivittatus* (Coq.) with *Dirofilaria immitis* in central Iowa. J. Parasitol. 62:276–280.

Erekson, N. P. 1991. Utah heartworm in dogs study 1991. Proc. Utah Mosq. Abatement Assoc. 44:31–33.

Grieve, R. B., J. B. Lok and L. T. Glickman. 1983. Epidemiology of canine heartworm infection. Epidemiol. Rev. 5:220–246.

Hatch, G. L. and S. L. Dickson. 1991. *Aedes sierrensis*, a new problem for Salt Lake City. Proc. Utah Mosq. Abatement Assoc. 44:34–35.

Hawley, W. A. 1985. A high-fecundity aedine: factors affecting egg production of the western treehole mosquito, *Aedes sierrensis* (Diptera: Culicidae). J. Med. Entomol. 22:220–225.

Lewandowski, H. B., Jr., G. R. Hooper and H. D. Newson. 1980. Determination of some important natural potential vectors of dog heartworm in central Michigan. Mosq. News 40:73–79.

Marshall, M. 1990. Canine heartworm infection. Proc. Utah Mosq. Abatement Assoc. 43:17–18.

Muller, R. L. and D. A. Denham. 1974. A field technique for the recovery and preservation of infective filarial larvae from their vectors. Trans. R. Soc. Trop. Med. Hyg. 68:8–9.

Nielsen, L. T., J. H. Arnell and J. H. Linam. 1967. A report on the distribution and biology of tree hole mosquitoes in the western United States. Proc. Calif. Mosq. Vector Control Assoc. 35:72–76.

Sears, B. W., G. L. McCallister and J. C. Heideman. 1980. *Dirofilaria immitis* in west-central Colorado. J. Parasitol. 66:1070.

Walters, L. L. and M. M. J. Lavoipierre. 1982. *Aedes vexans* and *Aedes sierrensis* (Diptera: Culicidae): potential vectors of *Dirofilaria immitis* in Tehama County, northern California, USA. J. Med. Entomol. 19:15–23.

Walters, L. L. and M. M. J. Lavoipierre. 1984. Landscape epidemiology of mosquito-borne canine heartworm (*Dirofilaria immitis*) in northern California, USA: I. Community-based surveys of domestic dogs in three landscapes. J. Med. Entomol. 21:1–16.

Weinmann, C. J. and R. Garcia. 1974. Canine heartworm in California, with observations on *Aedes sierrensis* as a potential vector. Calif. Vector Views 21:45–50.